QPatch HTX, we demonstrate that the QPatch also can be used as an efficient screening tool for ligand gated ion channels. In this study, we have tested 3 different ion channels in a screening assay on QPatch HTX: GABA $\alpha1\beta1\gamma2$ (γ-amino butyric acid receptor), ASIC a1 (acid sensing ion channel) and nAchR $\alpha1$ (nicotinic acetylcholine receptor). These assays each have their own unique set of challenges: Low functional expression, extremely high elicited current amplitudes, and fast desensitization respectively. In this poster we present data to compare throughput and assay quality between the QPatch HTX in multi-hole mode and the classic single-hole mode in a screening scenario.

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Subunit Oligomerization of Human ASIC1a in Xenopus Laevis Oocytes

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The crystal structure of the chicken Acid Sensing Ion Channel 1 (ASIC1) shows a homotrimeric channel complex. Because of conflicting results regarding the subunit stoichiometry of other members of the ASIC ion channel family, we have determined the mass of the functional human ASIC1a channel complex expressed in *Xenopus* oocytes.

We have used sulfhydryl crosslinkers or oxidizing agents to stabilize the native oligomeric ASIC1a complex. In the cut-open oocyte recording system, the intracellular perfusion of 2-20 mM sodium tetrathionate (NaTT) did not affect hASIC1a activity. Western blot analysis shows that NaTT induces a shift in the molecular weight of ASIC1a from the monomeric 70 kDa, to higher molecular weights of 140 and 280 kDa. ASIC1a purification after surface biotinylation and crosslinking shows that the major form of ASIC1a at the plasma membrane corresponds to a 280 kDa channel complex. The use of different sulfhydryl crosslinkers allowed to stabilize on Western blot oligomeric ASIC1a complexes of 280, 210 and 140 kDa corresponding to tetramers, trimers and dimers. This subunit crosslinking of the ASIC1a complex depends on cysteines in the C-terminus of the protein. We confirmed by size exclusion chromatography the presence of a hASIC1a channel complex of a mass corresponding approximatively to 280 kDa.

In *Xenopus* oocytes sulfhydryl crosslinkers or oxidizing reagents stabilized a hASIC1a complex of 280 kDa without affecting channel activity; the mass of this channel complex cannot simply account for a trimer. The C-terminus of ASIC1a subunits is involved in subunit-subunit interactions and may be important for the oligomerization of the functional channel complex.

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Identifying an Etomidate Binding Site in Heterologously Expressed Human Alpha1/Beta3 $GABA_A$ Receptors ($GABA_AR$) Using Photoactive Etomidate Analogs

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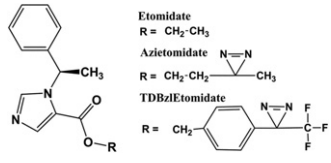
The $GABA_AR$ is a target for general anesthetics, which act as positive allosteric modulators at clinical doses. Previously, in a heterogeneous mixture of $GABA_ARs$ isolated from bovine brain, [^{3}H]azietomidate photolabeled αmMet-236 and βnMet-286 (m = 1, 2, 3 or 5; n = 1, 2 or 3) in the αM1 and βM3 transmembrane helices. To resolve uncertainties inherent in using natural sources of receptor, we used stable tetracycline-inducible HEK293 cells overexpressing $\alpha1\beta3$ $GABA_ARs$. Furthermore, to explore the proposed site in more detail, we used two photoactivable etomidate derivatives: [^{3}H]azietomidate, an aliphatic diazirine, and [^{3}H]TDBzlEtomidate, an aryl diazirine with a broader amino acid side chain reactivity profile (Figure). Purified recombinant human $\alpha1\beta3$ $GABA_ARs$, functionally reconstituted into detergent-asolectin, were photolabeled in the presence of GABA $\pm$ etomidate. [^{3}H]Azietomidate and [^{3}H]TDBzlEtomidate photolabeled β3Met-286 and α1Met-236, and [^{3}H]TDBzlEtomidate photolabeled β3Val-290, one helical turn below β3Met-286. These observations prove that the photolabeled β- and α-subunits belong to a single oligomer and strengthen the hypothesis that the three photolabeled residues all belong to a single site within the β-α subunit interface. (*S.S.J. and Z.D. contributed equally; supported by GM58448).



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A conserved Asparagine Between Loops A and E of Anion Ligand-Gated Channels is Critical for Gaba(A) Receptor Function

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Cys-loop ligand-gated ion channels are pentameric transmembrane proteins that are essential for the regulation of neuronal excitability. In synaptic GABA(A) receptors, residues in the two α-β+ interfaces are known to define the GABA binding site while homologous residues in the γ-α+ interface define the classical high-affinity benzodiazepine binding site. Very little however, is known about the structure and function of the remaining two interfaces: β-α+ and β-γ+. Since the β- interface is common to both, we chose to investigate the role this interface plays in receptor function, using the tools of structure-homology modeling, site-directed mutagenesis, transient expression of receptors in HEK-293 cells and whole-cell patch-clamp electrophysiology. In this study, we focused on a non-canonical loop domain (NCLD) that links the canonical loops A and E, also known as the β-strand 5-β- strand 5' linker. Homology models predict that the N-terminal end of this loop flanks a large inter-subunit lumen-accessible crevice. An alanine screen of the NCLD revealed 4 positions within the domain that are critical for receptor activation. Of particular interest was a highly-conserved asparagine: β2(N113). Our data indicate that mutations at this position results in reduced receptor sensitivity to GABA (increased EC_{50}), impaired receptor gating (reduced partial agonist efficacy) and perturbed ion flow (increased rectification). Interestingly, the change in rectification was reversed by the addition of positive allosteric modulators such as propofol and pregnanolone. Taken together, our observations demonstrate the importance of the non-binding interfaces in controlling receptor function.

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Etomidate Binding Interactions in $GABA_A$ Receptor Defined with Cysteine Substitutions

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The general anesthetic etomidate exerts its major clinical actions through potentiation of $GABA_A$ receptor activation. $GABA_A$ receptors are pentameric, usually consisting of combinations of alpha, beta, and gamma subunits. A photoreactive structural analog of etomdiate ([^{3}H]azietomidate) labels amino acids on transmembrane domains in both α (Met236) and β (Met286) subunits. Consistent with receptor structural homology models based on *Torpedo* nicotinic acetylcholine receptor, the labeling results suggests the presence of two interfacial anesthetic binding sites per $GABA_A$ receptor located at the transmembrane β/α subunit interface. However, azietomidate may fail to label other important binding determinants. In particular, β subunit reside 265 is predicted to be near the etomidate site, and $\beta2/\beta3$Asn265 mutations profoundly affect etomidate sensitivity. To determine if specific residues are within the etomidate site, we tested whether etomidate protects Cysteines substituted at these residues from sulfhydryl modifying reagents. In human $\alpha1\beta2\gamma2$L background, $GABA_A$ receptors with the α1M236C and α1L232C mutations displayed etomidate sensitivity similar to wild-type in electrophysiological experiments, while receptors with β2M286C and β2N265C mutations largely eliminated etomidate effects. All of these Cysteine substituted receptors were modified by p-chloromercuribenzenesulfonate (pCMBS), which altered electrophysiological properties. Using allosteric GABA/etomidate co-agonist gating models for each mutant, we identified protection conditions where the mix of receptor states was similar to control modification conditions and etomidate site occupancy was expected to be high. In the presence of etomidate, the apparent rate of pCMBS modification at both α1M236C and β2M286C was reduced more than ten-fold and was reduced two-fold at α1L232C. In contrast, pCMBS modification of β2N265C was unaffected by high concentrations of etomidate. These results suggest that etomidate sterically interacts strongly with α1Met236 and β2Met286 and weakly with α1Leu232, but does not interact with β2Asn265.

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A novel Polymorphism Linked to Epilepsy Encoding a Missense Mutation in the Pre-M1 Region of a6 Subunits Alters the Gating, but not Trafficking, of GABAa Receptors

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Charged residues in the pre-M1 region of Cys-loop ligand-gated ion channels affect current activation, desensitization and deactivation, suggesting that these interface residues may be involved in coupling agonist binding to channel gating. Mutations that affect function and/or expression of $GABA_A$ receptors (GABARs) have been associated with idiopathic generalized epilepsies (IGEs). The *Parallel Sequence Profiling of Ion Channels in Epilepsy Project* at Baylor College of Medicine funded by NINDS and NHGRI identified a novel single nucleotide polymorphism encoding a missense mutation, Q237R, in the GABAR α6 subunit in a patient with an IGE (http://www.hgsc.bcm.tmc.edu/project-medseq-r-ionchannel.hgsc). Homology modeling suggested that Q237R is located within the β10-M1linker or "pre-M1" segment of the N-terminal α6 subunit extracellular domain where it